

QAM-I-103

## Operation and Calibration of the UV-VIS Spectrophotometer

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Laboratory Manager

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*Texas Institute for Applied Environmental Research*

## Operation and Calibration of the UV-VIS Spectrophotometer

**1. Applicability**

This procedure applies to the operation and calibration of the Beckman Coulter DU-640 UV-VIS, Shimadzu UV-2450 (manual mode) and PerkinElmer Lambda 365 spectrophotometers located in the chemistry laboratory of the Texas Institute for Applied Environmental Research (TIAER), Tarleton State University, Stephenville, TX. This procedure shall be performed prior to any analysis using one of the spectrophotometers

**2. Purpose**

The purpose of this procedure is to provide a method for operation and calibration of the DU-640, UV-2450 and Lambda 365 spectrophotometers. By utilizing appropriate chemistry SOPs to perform the standard calibration for each set of samples, the analyst eradicates anomalies due to reagent mixture variations, sample preparation variations, and instrument sensitivity fluctuations. The operation of the instrument allows the analyst to determine the concentration of various chemical species which are dissolved in water samples or extracts analyzed by the TIAER chemistry laboratory.

**3. Definitions**

- 3.1. Automated Sipping Device- A device used in conjunction with the instrument serving as a sample container-to-photometric cell sample delivery conduit.

**4. Equipment, Reagents, and Standards****4.1. Equipment**

- 4.1.1. Beckman Coulter DU-640 spectrophotometer
  - 4.1.1.1. DU600/7000 Data Capture/Network Software ver. 2.2
  - 4.1.1.2. DU600/7000 File Utility Software ver. 1.2
- 4.1.2. Shimadzu UV-2450 spectrophotometer
  - 4.1.2.1. Shimadzu UV Probe Software ver. 2.33
- 4.1.3. PerkinElmer Lambda 365 spectrophotometer
  - 4.1.3.1. PerkinElmer UV WinLab Software ver. 7.1.0.68

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- 4.1.4. 10-mm square sided silica or quartz cuvette (for uv region) or 10-mm square sided glass or plastic cuvette (for the visible wavelength region); may be flow-through or manual pour cells
- 4.1.5. 100-mm tubular (or square-sided) cuvette, or other sizes
- 4.1.6. Cuvette holder (adapted for size of cuvette)
- 4.1.7. Automated sipping device or autosampler (if applicable)
- 4.1.8. Sample tubes or other glassware appropriately sized to serve as mixture vessels for water samples or extracts and reagents
- 4.1.9. Autosampler boats
- 4.2. Reagents
  - 4.2.1. Deionized Water (DI)
  - 4.2.2. Color reagent solutions- contain all color or complexing reagents, sample solvents, and other chemical species which will be added to the samples prior to analysis by the uv-vis instrument. Refer to the appropriate chemical SOP for specific solutions and uses. Non-color reagents are also used in organic extractions and sample preparation.
  - 4.2.3. Cleaning solution for automated sipper and cell- add 5 drops Triton-X 100™ and 5g NaOH to 500 mL DI H<sub>2</sub>O and dissolve.
- 4.3. Standards
  - 4.3.1. The standards required for the analysis of samples are determined by the analyte. Prior to performing any analytical procedure using the uv-vis spectrophotometer, the standard operating procedure for chemical analysis are reviewed to determine all of the required parameters and standards.

## **5. Procedure**

- 5.1. Beckman Coulter DU-640 (primarily for determination of OPO4P and as a backup to the other instruments)
  - 5.1.1. Powering Up
    - 5.1.1.1. The instrument may be turned off overnight.
    - 5.1.1.2. Turn on the instrument with the power switch located on the right-hand side of the back of the instrument. The display should illuminate, and a message window with "Executing Power Up Diagnostics" should be displayed.

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- 5.1.1.3. In a few minutes a table indicating the Power Up Diagnostics will be displayed. The table should indicate that the parameters “RAM Option” and “Programmability” are “Not Installed” and that “RS232 Ports 3 and 4” is “Installed.” The word “Passed” should be indicated for all other parameters.
  - 5.1.1.4. If any parameter fails, flush the flow cell with cleaning solution and DI water and turn the instrument off. Repeat power-up steps. Consult manual for troubleshooting, if necessary. Continue when the instrument successfully completes power up diagnostics.
  - 5.1.1.5. Move the cursor arrow to the upper right hand corner of the Power Up Diagnostics table to the word quit and click on it to exit.
- 5.1.2. Calibration
- 5.1.2.1. Turn on the appropriate source by clicking on the [VIS OFF] or [UV OFF] on the menu bar. The icon selected should turn red and change to [VIS ON] or [UV ON] to indicate that the source is on.
  - 5.1.2.2. Allow the instrument and source to warm up and stabilize for approximately 30 minutes.
  - 5.1.2.3. Select the “Fixed Wavelength” mode.
  - 5.1.2.4. Prepare a calibration blank with DI water or extracting solution and all reagents that will be used in the samples according to the proper procedure.
  - 5.1.2.5. The instrument should be in absorbance mode. Mode can be changed by clicking the icon next to the word “mode.” Clicking will toggle the instrument between absorbance “Abs” and percent transmittance “%T”.
  - 5.1.2.6. Click on the field to the right of the “ $\lambda$ ” and input the proper wavelength for the analyte being tested.
  - 5.1.2.7. Draw the blank into the flow cell by inserting the sipper tube into the culture tube and pressing the fill button on the pump.

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- 5.1.2.8. Click on the “blank” icon on the menu bar. When the “Reading Blank” box disappears the instrument is calibrated and ready for use.
- 5.1.3. Pre-operation Checks
  - 5.1.3.1. Prior to performing any analytical operations, the analyst verifies proper connection between the instrument and all output devices.
  - 5.1.3.2. If the system is connected to an automatic sipping device, check the connections between the device and the instrument also.
  - 5.1.3.3. Once all connections have been checked, sample analysis is ready to be performed.
- 5.1.4. Operation
  - 5.1.4.1. Power up instrument as described above.
  - 5.1.4.2. Calibrate the instrument as described above.
  - 5.1.4.3. Click on the field to the right of the “Factor” and input the proper factor.
  - 5.1.4.4. Using the proper vials as reaction containers, prepare the samples and standards as indicated in the standard operating procedure for the chemical analysis.
  - 5.1.4.5. Normally the analytical order is set by placing a blank solution in the first slot, then following with the standards, samples and quality control aliquots, which include duplicates and spikes.
  - 5.1.4.6. Use the complete set of standards listed in the standard operating procedure for chemical analysis immediately after the blank.
  - 5.1.4.7. An initial calibration verification and initial blank verification should be performed at the beginning of an analytical run and a continuing calibration verification and continuing calibration blank check are placed at the end of each QC batch. Other verification such as laboratory control standards or limit of quantification standards may be used if required by project.
  - 5.1.4.8. Load the sample boat onto the auto-sampling device.

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- 5.1.4.9. Click on the “Read Samples” icon to begin analysis and allow the instrument and auto-sampler to complete the run.
  - 5.1.4.10. When the run is complete open the “1. Get Data” icon on the auxillary computer. This will bring up a box, click the “activate” button. Open the “SAVECLEAR” icon on the instrument screen. Change the file name to “DATAMMDD,” where MM is the month and DD is the day, and click “O.K.” Choose “O.K.” and overwrite both files. Exit the box on the auxillary computer. All further steps will be with the auxillary computer only.
  - 5.1.4.11. Open the “2. Change Data” icon. Open the file “DATAMMDD.duf” or “DATAMMDD” file in the directory: C:\Program Files\DU WinConnection\DU Data Capture\Data\Fix\_Data. Change the data to Excel format and save as “data.” The old file will be written over.
  - 5.1.4.12. Open the “3. Data” icon and type in standard designations and sample #s in Sample ID column. Print the file and affix to the Personal Logbook.
  - 5.1.4.13. If desired, automatically transfer the data file to ESDMS or data management system.
  - 5.1.4.14. Data may also be entered manually into ESDMS system.
  - 5.1.4.15. Flush ethanol through the system, followed by DI. Other cleaning solutions may be used on occasion. Refer to specific SOP for instructions.
  - 5.1.4.16. If finished, turn off the light source and clean area.
- 5.2. Shimadzu UV-2450- Manual only, no autosampler used at this time (primarily used for chlorophyll and pheophytin analyses, low level orthophosphate P and NO<sub>3</sub>N and as a backup to the DU-640)
- 5.2.1. Power up the instrument and the computer.
  - 5.2.2. Start the UVProbe 2.33 software from the computer icon.
  - 5.2.3. Install the correct cell holder for the application (refer to analytical SOP for details). Leave the cell slot blank.

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- 5.2.4. Ensure that the “Photometric” view is open, then click “Connect” to establish the link between computer and instrument. This may take several minutes.
  - 5.2.5. If connection window shows errors, troubleshoot according to the User’s Manual. Problems may include power not being on, blocked light path or light source not working.
  - 5.2.6. Once all tests have passed and a connection is established, click “OK”.
  - 5.2.7. Click the “λ Go to WL” and enter the wavelength desired.
  - 5.2.8. Fill the required cell with blank reagent or reacted blank. Use a Kimwipe to gently wipe down the cell light path and hold up to look through at light. Ensure no droplets or smudges are present. Wipe until clean. DI water or ethanol may be needed to clean the cell ends.
  - 5.2.9. Place the cell in the holder and click “Auto Zero”. Repeat for each wavelength used per analytical SOP.
  - 5.2.10. The program takes continuous readings for subsequent measurements of samples and standards in the cell. Allow the reading to stabilize to 3 decimal places and take the best reading for the 4<sup>th</sup>.
  - 5.2.11. Power off instrument and computer when complete and clean up cell and work area.
  - 5.2.12. Other developmental methods such as multiwavelength scans and timed reading may be performed for informational studies and research. Consult the User’s Manual for these operations. Included herein is only the simple, manual operation of the instrument.
- 5.3. Perkin Elmer Lambda 365 – Manual only, no autosampler used at this time (primarily used for chlorophyll and pheophytin analyses, low level orthophosphate P and NO<sub>3</sub>N and as backup to the DU-640)
- 5.3.1. Power up the instrument and the computer.
  - 5.3.2. Start the UV WinLab software from the icon on the desktop and wait for the computer to connect to the instrument.



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- 5.3.3. Click on "Methods" on the "Folder List" and choose the desired method from the menu, such as "Chla/Phea," or create a new method.
- 5.3.4. Wait for the instrument to initialize. A summary of the checks will appear when this is complete. Click "OK" to proceed. Wait until "Idle" appears in the top left corner before proceeding with analysis.
- 5.3.5. Input the sample info.
- 5.3.6. Install the correct cell holder for the application (refer to the analytical SOP for details).
- 5.3.7. Fill the required cell with blank reagent or reacted blank. Use a Kimwipe to gently wipe down the cell light path and hold up to look through at light. Ensure no droplets or smudges are present. Wipe until clean. DI water or ethanol may be needed to clean the cell ends.
- 5.3.8. Place the cell in the holder and click "Auto Zero".
- 5.3.9. Discard blank and fill the cell with the first sample or QC aliquot.
- 5.3.10. Press "Start" to begin the run.
- 5.3.11. Continue with remaining samples.
- 5.3.12. Power off instrument and computer when complete and clean up cell and work area.
- 5.3.13. Other developmental methods such as multiwavelength scans and timed reading may be performed for informational studies and research. Consult the User's Manual for these operations. Included herein is only the simple, manual operation of the instrument.

## 6. Quality Control and Safety Aspects

- 6.1. All aspects of this procedure shall comply with QAM -Q-101, "Laboratory Quality Control", and QAM-S-101, "Laboratory Safety".
- 6.2. Quality Control
  - 6.2.1. The analyst always uses matched cuvettes, if using more than one cuvette, when performing analysis with the uv-vis spectrophotometer. This ensures the pathlength parameters will be equivalent and the measurements are accurate and reproducible.



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- 6.2.2. The preparation of all samples, standards, and blanks are carried out using identical methods and preparatory equipment. This eliminates the majority of errors which may be introduced during sample solution preparation.
- 6.2.3. The auto-sampler, cuvettes, and all associated equipment are cleaned and rinsed thoroughly with DI water prior to and following use. Other cleaning solutions may be required on occasion at the direction of the Laboratory Manager.
- 6.2.4. All instrument maintenance performed on the spectrophotometer is documented in the Maintenance Logbook, QAM-Q-103-1.
- 6.2.5. Any major modification or parts replacements initiates a new Demonstration of Performance in accordance with QAM-Q-101, "Laboratory Quality Control", to ensure the instrument is functioning properly prior to data generation.
- 6.2.6. The DU-640 retains calibration factors and only needs to be re-calibrated at the direction of the Laboratory Manager. Calibration information is kept with each analytical run record.
- 6.3. Safety
- 6.3.1. The solvents or complexing reagents which are used during analysis of water samples or extracts may be toxic or acidic. The analyst always consults the MSDS files if he or she has any question as to the safe handling of any reagent required by the standard operating procedure for chemical analysis.

## 7. References

- 7.1. DU® SERIES 600 SPECTROPHOTOMETER, OPERATING INSTRUCTIONS, MANUAL, Beckman Instruments, Inc, 1992, Fullerton, CA, Manual 514531B.
- 7.2. User's Manual, UV-2450, Shimadzu Corporation, 2008, Kyoto, Japan.
- 7.3. Lambda 365 Users Guide, PerkinElmer, Inc., March 2020, Part Number 09931249.
- 7.4. National Environmental Laboratory Accreditation Conference (NELAC) standards, TNI, 2016.
- 7.5. TIAER Quality Assurance Manual, QAM-Q-100, latest revision.

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8. **Attachments**

None

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